The relationship between cognitive function and high-resolution diffusion tensor MRI of the cingulum bundle in multiple sclerosis


Abstract

Background: Imaging can provide noninvasive neural markers of disease progression in multiple sclerosis (MS) that are related to behavioral and cognitive symptoms. Past work suggests that diffusion tensor imaging (DTI) provides a measure of white matter pathology, including demyelination and axonal counts. Objectives: In the current study, the authors investigate the relationship of DTI measures in the cingulum bundle to common deficits in MS, including episodic memory, working memory, and information processing speed. Methods: Fifty-seven patients with MS and 17 age- and education-matched controls underwent high-spatial resolution diffusion scans and cognitive testing. Probabilistic tracking was used to generate tracks from the posterior cingulate cortex to the entorhinal cortex. Results: Radial and axial diffusivity values were significantly different between patients and controls ($p < 0.031$), and in patients bilateral diffusion measures were significantly related to measures of episodic memory and speed of processing ($p < 0.033$). Conclusions: The tractography-based measures of posterior cingulum integrity reported here support further development of DTI as a viable measure of axonal integrity and cognitive function in patients with MS.

Keywords: Multiple sclerosis, cognition, diffusion tensor imaging, episodic memory, disease progression, cingulate gyrus

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The cingulum bundle is a large tract of WM fibers originating in the cingulate cortex, projecting to the entorhinal cortex (EC) of the temporal lobe. Through the cingulum bundle, the posterior cingulate gyrus has multiple reciprocal connections with the hippocampus, a structure involved in episodic memory that shows structural and functional changes in patients with MS. Diffusion measures in the posterior cingulum bundle (PCB) have shown relationships to verbal and visual spatial episodic memory, executive function, and working memory in various patient populations and in healthy controls.

In this study, probabilistic fiber tracking is used to examine the integrity of the PCB between the posterior cingulate cortex (PCC) and the EC. Resultant diffusion measures are compared to performance on measures of verbal and visual spatial episodic memory, information processing speed, and working memory. Radial (RD) and axial diffusivity (AD) are commonly used diffusion tensor-derived scalars. Changes in these diffusivities in WM have been shown to relate to demyelination and axonal damage, both processes known to be involved in MS. The importance of the cingulum bundle in memory and the strong reciprocal connections with the hippocampal formation lead us to hypothesize that measures of RD and AD in the PCB will be increased in patients with MS as compared to controls. Further, we hypothesize that RD and AD will be related to performance on the California Verbal Learning Test-II (CVLT), which measures verbal episodic memory, and the Brief Visuospatial Memory Test-R (BVMT), measuring visual spatial episodic memory, and the Paced Auditory Serial Addition Test (PASAT), which measures speed of processing, attention, calculation ability, and working memory.

To determine the specificity of our findings, we also assess the relationship of diffusion measures in a portion of the corticospinal tract (CST) (the posterior limb of the internal capsule (PLIC)) to cognitive measures. The CST is not related to memory performance, and we hypothesize that RD and AD in this region will not be related to cognitive function. Mean diffusivity (MD) and fractional anisotropy (FA) are tensor-derived scalars that are commonly reported in the literature. Although these are, in a sense, derived from RD and AD, we report results for these measures for completeness and compatibility with prior literature.

Methods

Sample

The original dataset was composed of 64 patients with MS and 20 controls with complete DTI scans. Prior to data analysis, the sub-sample used in this research was selected with the goal of creating an age- and education-matched sample that included as many participants as possible. From this original dataset, seven patients with MS were excluded, all the oldest participants with the lowest levels of education (two males; mean (standard deviation) age: 52.0 (6.1) and education: 12.0 (0.8)). Three control participants were excluded, all the youngest with the highest levels of education (three males; mean (standard deviation) age: 34.3 (2.5) and education: 20.0 (0.0)). The final sample included 57 patients with MS and 17 age- and education-matched controls.

MRI

All data were acquired under a Cleveland Clinic Institutional Review Board-approved protocol. All participants were fitted for a bite bar to restrict head motion during scanning and were then scanned using a 12-channel receive-only head array on a Siemens TIM Trio 3 tesla scanner (Siemens Medical Solutions, Erlangen, Germany). The following scans were performed:

- Scan 1: Whole-brain T1. T1-weighted inversion recovery turboflash (magnetization-prepared rapid acquisition gradient echo (MPRAGE)): 120 axial slices; slice thickness = 1.2 mm; field-of-view (FOV) 256 × 256 mm²; inversion time (TI)/echo time (TE)/repetition time (TR)/flip angle (FLA) = 900/1.71/1900 ms/8; matrix 256 × 128; receiver bandwidth (BW) = 62 kHz.
- Scan 2: Sampling perfection with application optimized contrast using different FLA evolutions (SPACE) three-dimensional (3D) T2: 144 sagittal slices; slice thickness = 1.2 mm; FOV = 256 × 224 mm²; inversion time (TI)/echo time (TE)/repetition time (TR)/flip angle (FLA) = 900/1.71/1900 ms/8; matrix 256 × 128; receiver bandwidth (BW) = 62 kHz.
- Scan 3: SPACE 3D fluid-attenuated inversion recovery (FLAIR): 144 sagittal slices; slice thickness = 1.2 mm; FOV = 256 × 224 mm²; matrix = 256 × 224; 6/8 partial Fourier acquisition; TE/TR = 528/3200 ms; FLA mode; generalized auto-calibrating partially parallel acquisitions (GRAPPA) factor = 2; 24 reference lines; BW = 434 Hz/pixel.
- Scan 4: Whole-brain fieldmap. Axial gradient recalled echo: 32 axial slices; slice thickness = 4 mm; FOV = 256 × 256 mm²; matrix = 64×64; TE1/TE2/TR/FLA 4.89/7.35/388 ms/60 degrees; BW = 260 Hz/pixel.
• Scan 5: High angular resolution diffusion imaging (HARDI). Single-shot echo-planar imaging readout; FOV = 192 × 192 mm²; matrix = 192 × 192; 45 1 mm thick slices; TE/TR = 90/7700 ms; 6/8 partial Fourier factor with GRAPPA acceleration factor = 2; readout BW = 930 Hz/pixel; 71 directions; two averages; 8 b = 0 acquisitions per average. Diffusion weighting was achieved with a Stejskal-Tanner scheme at high angular resolution and with multiple diffusion weightings (72, 32, 8, and 9 image volumes acquired at b = 750, 333, 83, and 0 s/mm², respectively).

HARDI postprocessing
Motion correction was performed with an iterative algorithm and the diffusion tensor was calculated using a standard log-linear fit.\textsuperscript{18,19} Eigenvalues of each tensor were then used to calculate AD, RD, FA, and MD. The fiber orientation distribution (FOD) was calculated from the b = 750 s/mm² images, using regularized spherical deconvolution as the basis for probabilistic tractography.\textsuperscript{20}

Regions of interest (ROIs)
For each participant, the MPRAGE was transformed to Talairach space using AFNI.\textsuperscript{21} EC ROIs were drawn manually on the individual participant MPRAGE in Talairach space and were restricted to coronal slices 10P–30P. PCC ROIs consisted of a 6 mm sphere placed on the Talairach-transformed MPRAGE, according to the coordinates (–12, –42, 36) given in Greicius et al.\textsuperscript{22} The PLIC was drawn bilaterally in Talairach space to ensure similar coverage for all participants. All ROIs were transformed to individual participant space and checked for accuracy. PCC and EC ROIs (Figure 1) and PLIC ROIs (Figure 2) are shown in representative individuals. Because of the high level of atrophy in some of the patient sample, multiple steps were taken to ensure accurate registration between anatomical and diffusion scans. First, the mean b = 0 image from the HARDI scan was spatially unwrapped using the FSL tool FLIRT,\textsuperscript{23,24} allowing for more accurate registration between the DTI and anatomic images. For each participant, the spatial transformation matrix between anatomic images and DTI was calculated using the AFNI routine align_epi_anat.py and the resulting registration was visually inspected for accuracy. All ROIs were spatially registered to DTI space and again checked for placement accuracy. PLIC ROIs were also inspected on color FA maps to verify accurate placement.

WM mask and volumetric analysis
To ensure that DTI measures were from WM, FSL was used to derive a WM mask from the MPRAGE, T2, and FLAIR. The WM mask, w(v), was spatially registered to DTI space and visually inspected for accurate registration. For our analysis, w(v) = 1 for all voxels (v) that were determined with high probability to contain WM and w(v) = 0 otherwise. For group comparisons, whole-brain measures of WM and gray matter (GM) and a scaling factor used as a correction for head size were estimated using FSL.

Probabilistic tracking
More than 1 million tracks were generated from seed regions placed in the bilateral PCC. Of

Figure 1. A representative PCB pathway (yellow), and ROIs for the EC (green) and PCC (blue). PCB: posterior cingulum bundle; ROIs: regions of interest; EC: entorhinal cortex; PCC: posterior cingulate cortex.

Figure 2. Representative ROIs for the PLIC (red) overlaid on a color FA map. ROIs: regions of interest; PLIC: posterior limb of the internal capsule; FA: fractional anisotropy.
the generated tracks, only those that terminated in a
target region placed in the EC were saved. The num-
ber of saved tracks intersecting each voxel was then
used to generate track density maps. Typically,
approximately 4000 tracks passed through the target
ROI; this number was determined to be sufficient to
produce track density maps with dynamic range
such that stable pathway-dependent measures could
be obtained.

Pathway-based DTI measures were produced by
using a weighted mean:

\[
\langle D \rangle = \frac{\sum \sum_{v} D(v) \cdot w(v) \cdot WM(v)}{\sum \sum_{v} w(v) \cdot WM(v)}
\]

in which \( D(v) \) is the particular tensor-based value of
interest (e.g. RD) at voxel \( v \), and \( w(v) \) is the value
of the WM mask at voxel \( v \). The inner summation is
over all voxels \( v \) on track \( T \); the outer summation
is over all PCB pathway tracks \( T \). Note that the
effect of the above equation is to weight the tensor
value of a voxel by the number of times a generated
track passes through it. Thus, voxels with only a few
tracks passing through will count very little toward
the pathway tensor estimate, whereas tracks more
central to the path will be counted very highly. The
result is AD, RD, FA, and MD for every track.

Within individuals, left and right PCB track values
showed high agreement (intraclass correlation coef-
ficient > 0.77). Accordingly, the mean of each meas-
ure across the right and left tracks was taken for each
individual.

PLIC analysis
For each participant, mean and standard deviation
values for RD, AD, FA, and MD were calculated for
voxels that were included in the PLIC ROIs and the
WM mask. To ensure PLIC measures were as compa-
rable to PCB measures as possible, the mean of the
right and left PLIC diffusion values were taken as the
final measure.

Behavioral data
All participants completed a neuropsychological
assessment and several measures of cognitive func-
tion. A neurologist specializing in MS rated all
patients on the Expanded Disability Status Scale
(EDSS), and a composite Multiple Sclerosis
Functional Composite (MSFC) score was calculated
for each participant. Cognitive tests included:

1. CVLT—verbal episodic memory (sum of trials 1–5);
2. BVMT—visual spatial episodic memory (sum
   of trials 1–3);
3. Symbol Digit Modalities Test (SDMT)—pro-
   cessing speed, attention, and working memory
   (total score)\(^25\); and
4. PASAT—working memory, calculation, and
   speed of processing (total score, three-second
   administration).

Results
Fifty-seven patients with MS (44 relapsing–remitting,
13 secondary progressive) and 17 healthy controls
were scanned under the above imaging protocol. All
participants were right handed. Demographic inform-
ation and disease characteristics are detailed in Table 1.
Unpaired Student’s \( t \)-tests showed no differences in
age or level of education between patients and con-
trols. Scaled WM and GM volumes are reported in
Table 1. Both WM \( (p = 0.0063) \) and GM \( (p = 2 \times 10^{-4}) \)
volumes were smaller in patients with MS.

Behavioral results
Uncorrected results of cognitive measures for both
groups are shown in Table 1. Published norms were
used to correct raw scores for demographic variables.
The sum of trials 1–5 on the CVLT and total score on
the SDMT were both corrected for age and educa-
tion.\(^{15,25}\) The sum of trials 1–3 on the BVMT was cor-
rected for age,\(^{16}\) and total score on the PASAT was
corrected for education.\(^{26}\) Unpaired Student’s \( t \)-tests
were used to compare patient and control perfor-
mance. Patients scored significantly lower than con-
trols on all measures \( (p < 0.014) \). All comparisons
survived the false discovery rate (FDR) correction.\(^{27}\)

Diffusion results
Table 2 summarizes group diffusion measures for the
PCB and PLIC, and Figure 1 shows PCB tracks in a
representative individual. Unpaired Student’s \( t \)-tests
showed that bilateral PCB RD \( (p = 0.0001) \) and AD
\( (p = 0.0103) \) were significantly higher in patients with
MS than in controls. All comparisons survived the
FDR correction. There were no differences between
patient and control PLIC measures (Table 2). There
were no sex differences in diffusion measures in either
patients or controls.

In patients, age-adjusted partial correlations were
used to assess the relationship of diffusion measures
to disease characteristics. PCB measures were not
related to the MSFC. PCB RD was related to EDSS ($r = 0.347, p = 0.009$) and AD was related to disease duration ($r = 0.342, p = 0.010$), but these results did not survive the FDR procedure. PLIC AD and MD were related to the MSFC ($r = -0.293, p = 0.029$ and $r = -0.346, p = 0.009$, respectively), but this result also did not survive the FDR procedure.

Table 3 shows age-adjusted partial correlations between diffusion measures and cognitive performance. BVMT and SDMT performance were significantly related to RD in the PCB ($p < 0.001$), and SDMT performance was significantly related to RD in the PLIC ($p = 0.007$). Neither the CVLT nor the PASAT were related to diffusion measures. Multiple linear regression was used to further investigate the relationship between cognitive performance and PCB RD. Predictor variables included age and performance on the BVMT and SDMT. Only the BVMT had significant ($p = 0.035$) partial effects in the full model. This model accounted for 25% of the
Table 3. Linear partial correlation coefficient between cognition and bilateral PCB pathway diffusion measures, controlled for age, in 57 patients with MS.

<table>
<thead>
<tr>
<th></th>
<th>RD</th>
<th>AD</th>
<th>FA</th>
<th>MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVLT</td>
<td>−0.288</td>
<td>−0.203</td>
<td>0.231</td>
<td>−0.267</td>
</tr>
<tr>
<td>BVMT</td>
<td>−0.461</td>
<td>−0.311</td>
<td>0.358</td>
<td>−0.422</td>
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<tr>
<td>SDMT</td>
<td>−0.418</td>
<td>−0.285</td>
<td>0.342</td>
<td>−0.384</td>
</tr>
<tr>
<td>PASAT 3</td>
<td>−0.222</td>
<td>−0.147</td>
<td>0.173</td>
<td>−0.202</td>
</tr>
<tr>
<td>PLIC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CVLT</td>
<td>−0.233</td>
<td>−0.202</td>
<td>0.078</td>
<td>−0.284</td>
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<tr>
<td>BVMT</td>
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<td>−0.077</td>
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<tr>
<td>SDMT</td>
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<td>−0.247</td>
<td>0.181</td>
<td>−0.392</td>
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<tr>
<td>PASAT 3</td>
<td>−0.072</td>
<td>−0.203</td>
<td>−0.037</td>
<td>−0.189</td>
</tr>
</tbody>
</table>

Results in bold survive the false discovery rate (FDR) correction. *p < 0.01; **p < 0.005; ***p < 0.001; ****p < 0.0005; PCB: posterior cingulum bundle; PLIC: posterior limb of the internal capsule; CVLT: California Verbal Learning Test-II; BVMT: Brief Visuospatial Memory Test-Revised; SDMT: Symbol Digit Modalities Test; PASAT: Paced Auditory Serial Addition Test.

Discussion

We found significant differences between patients with MS and controls on all measures of diffusion in the PCB. In addition, RD was significantly related to performance on the BVMT and SDMT, and BVMT performance was a significant predictor of RD. PLIC measures did not show between-group differences, but RD was significantly related to performance on the SDMT.

Group differences in PCB diffusion measures are consistent with findings of axonal degradation and demyelination in MS damage.14 Our findings are in agreement with previously published reports of diffusion differences in ROI-based measures of the cingulum bundle in MS.28-30 and concur with a previous report of relationships between PCB diffusion and measures of both disease duration and EDSS.30 Lesions identified with conventional MR methods are not well correlated with clinic measures, and pathway-specific diffusion measures may be more sensitive to subtle changes in normal-appearing white matter (NAWM).1 DTI has been shown to detect focal lesions and lesion burden in NAWM, with both showing an increase in MD and a reduction in FA.31 Probabilistic tracking allows tracking in NAWM and through focal lesions, and our results suggest that track-based measures provide a fuller picture of WM damage compared to measures such as T2 lesion burden.

Our ROI-based PLIC measures did not show group differences, in contrast to previously published investigations of the CST.32-34 In a tract-based study of the CST, 59 patients with MS showed decreased FA compared to healthy controls.34 The PLIC FA reported in Table 2 is slightly higher than in that study and another tract-based CST analysis, though the standard deviations are similar.32,34 Our PLIC ROIs were carefully inspected to ensure anatomical accuracy, but it is possible that our ROI-based measure does not fully capture diffusion along the CST. Both tract-based CST studies showed a relationship between diffusion and EDSS.32,34 Though we did not find a relationship with EDSS, we did find relationships between PLIC diffusion and another measure of disability, MSFC score. Our sample appears to have a slightly truncated EDSS range compared to those used in the tract-based analyses, which may have influenced this result.

The relationships between diffusion values and cognitive performance in patients were only partially in line with our hypothesis. As predicted, performance on the BVMT, a measure of visual spatial episodic memory, was correlated with diffusion measures, particularly RD. BVMT was also the only significant predictor of RD in a regression using significant cognitive measures. The EC is known to play a role in visual spatial episodic memory, and a recent study of mild cognitive impairment (MCI) and early Alzheimer’s disease shows a relationship between diffusion measures in the PCB and visual memory.12 Verbal memory has also been related to diffusion measures in the posterior region of the cingulum bundle and EC in patients with MCI and Alzheimer’s disease.35 We anticipated a relationship with our measure of verbal episodic memory, the CVLT, but the correlation with RD did not survive a correction for multiple
comparisons. Surprisingly, we did not find a relationship between performance on the PASAT and diffusion measures. Previous studies have shown relationships between cingulum diffusion and performance on the PASAT in MS patients, though it should be noted that in both works the region of correlation was superior to the cingulum region analyzed in the current study.29,30 One limitation of this study is that of low disease burden in the patient sample. It is possible that the relationship of CVLT and PASAT performance to diffusion measures would strengthen with a greater range of disease burden, though work in healthy controls has shown a relationship between diffusion measures in the PCB and working memory and executive function, leading to expectations of a relationship to a measure such as the PASAT.13

Finally, we found a significant relationship between performance on the SDMT and RD in both the PCB and the PLIC. The SDMT is very sensitive to cognitive deterioration in MS and showed the greatest difference between patients and controls in this sample. We previously found a strong relationship between SDMT performance and diffusion measures,11 and it is possible that a change in information processing speed is an indicator of overall cognitive decline in MS, unrelated to track-specific changes.

In this study we observed a significant increase in PCB RD and AD in patients with MS. Further, patients showed a significant relationship between measures of visual spatial memory and information processing speed and diffusion of the PCB. Though this work includes only 57 patients, it does support continued development of DTI measures as a marker of cognitive function in patients with MS. It suggests that changes in specific abilities are associated with dysfunction in related tracts, and that information processing speed might be related to overall cognitive and neurofunctional decline. Future refinement and validation will require larger sample sizes, longitudinal follow-up, and the inclusion of validation cohorts.

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Conflict of interest
None declared.

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